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More than we bargained for: Zebra mussels transported amongst European native freshwater snails

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Abstract

The international pet trade is a major driver of non-native species spread, including species both sold in the trade, and organisms incidentally transported alongside. Here, we document the discovery of invasive zebra mussels, *Dreissena polymorpha*, in Germany, transported alongside a commonly traded garden pond snail and European native, *Viviparus viviparus*, ordered from a German pet website. We highlight that the trade poses yet another way in which zebra mussels and other invasive species can expand their invaded range into novel ecosystems. We call for stricter biosecurity enforcement towards sellers, and encourage raising awareness amongst customers to inhibit the further spread of invasive species through the pet trade.

Keywords

DNA sequencing, *Dreissena polymorpha*, hitchhikers, invasive species, pet trade, *Viviparus viviparus*

Introduction

The international pet trade has facilitated the movement of organisms around the world, and is deemed responsible for a third of all aquatic non-native species (Padilla and Williams 2004), with escapes and releases from the aquarium trade a major pathway for non-native freshwater species in Europe (Nunes et al. 2015). Difficulties

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surrounding regulation and enforcement (Patoka et al. 2018) are likely to increase as this global market continues to grow, with advances in technology increasing the availability of species from around the world, with websites and informal, peer-to-peer online marketplaces providing new purchasing options for customers (Olden et al. 2021). While the release and escape of traded species are often the focus of invasion ecologists (Kouba et al. 2021; Dickey et al. 2022), the risk of spreading "hitchhikers", i.e. fauna carried incidentally, has only recently received more interest (Duggan 2010). Indeed, recent studies have found the protozoan *Vorticella* sp. and a species of bdelloid rotifer associated with two species of atyid shrimps (Patoka et al. 2016), digenean larvae with the carnivorous gastropod *A. helena* (Stanicka et al. 2022), and an epibiont, *Diceratocephala boschmai*, on New Guinean ornamental *Cherax* crayfish (Lozek et al. 2021).

A high-profile example of an aquarium hitchhiker came in 2021, when zebra mussels (*Dreissena polymorpha*) were detected in 21 US states on aquarium moss balls that had been imported from Ukraine (United States Geological Survey Communications and Publishing 2021), and similar findings have emerged from Europe (Patoka and Patoková 2021). The zebra mussel is a Ponto-Caspian bivalve species that has colonized European and North American waters, and has been listed as one of the IUCN's 100 of the Worst Invasive Species (Lowe and Poorter 2000) due to its myriad economic (Connelly et al. 2007) and ecological impacts (Karatayev et al. 2002). By forming dense biogenic reefs, they compete with native unionids and zooplankton for planktonic food sources, and with fish for benthic space (Karatayev et al. 2002; Minchin et al. 2002). They also create hard-substrata in otherwise soft sediment environments, and affect water chemistry and clarity which in turn affects planktonic community, macrophyte coverage and food-web structure (Karatayev et al. 2002; Kirsch and Dzialowski 2012). With high byssal thread synthesis and attachment strength (Peyer et al. 2009), zebra mussels are capable of attaching to other organisms and boat hulls for overland transport and further spread (Collas et al. 2018). Here, we report the concerning arrival of zebra mussels amongst a delivery of a European native snail species, *Viviparus viviparus*, from an online pet store.

Methods and results

Discovery

Seventy-five *V. viviparus* (mean shell width ± standard error: 30.44 ± 0.39 mm; shell measured as per Fig. 2 in Jakubik and Lewandowski 2007) were ordered from a German online pet store (store name intentionally omitted). They arrived on the 8th April 2022, split across three plastic bags (n = 25 in each), in a polystyrene box. Upon arrival, they were taken to a climate control chamber (temperature 18 ± 1 °C), split into two 56 L glass aquaria holding tanks (60 cm × 30 cm × 30 cm length, width and height) containing 20 µm filtered freshwater and a filter, with thoroughly washed sand (1 cm deep) and white cockle shells for habitat. On the 9th April, two living freshwater mussels were found to be attached to two of the snails. These were subsequently measured

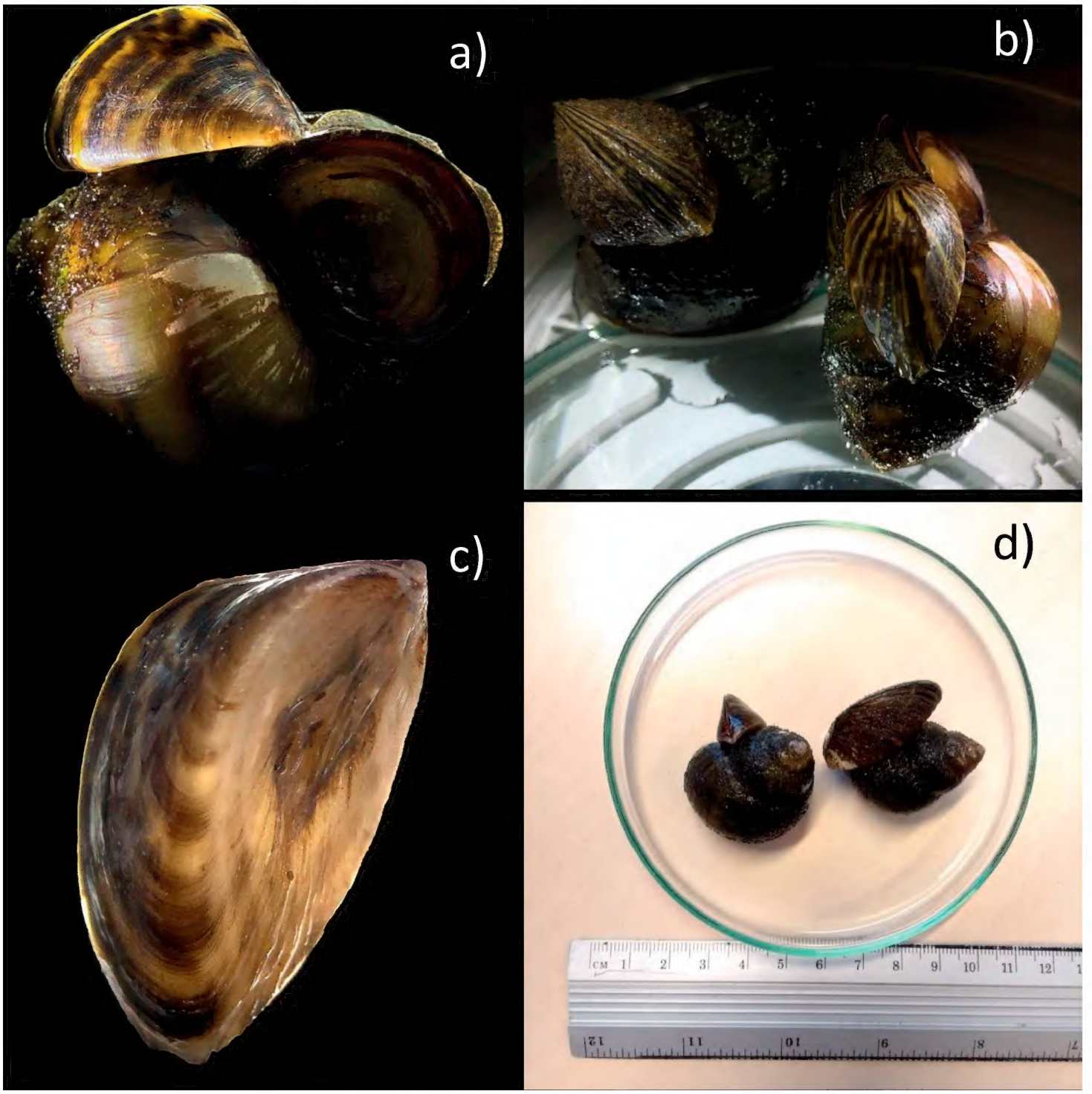

Figure 1. Zebra mussels, *Dreissena polymorpha*, found amongst ordered European pond snail, *Viviparus viviparus*.

with calipers (Mussel 1: length 30.7 mm, width 15.5 mm; Mussel 2: length 19.3 cm, width 11.0 mm – note both too large to have come from the laboratory water source), photographed (Fig. 1) and preserved in ethanol under refrigerated conditions for subsequent molecular identification.

DNA extraction, PCR and sequencing

DNA was extracted from the foot tissue of two mussel specimens using the DNeasy Blood & Tissue Kit (Qiagen, Germany) following the manufacturer's instructions. A fragment of the mitochondrial cytochrome *c* oxidase subunit I (*COI*) was amplified using primer pair LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994).

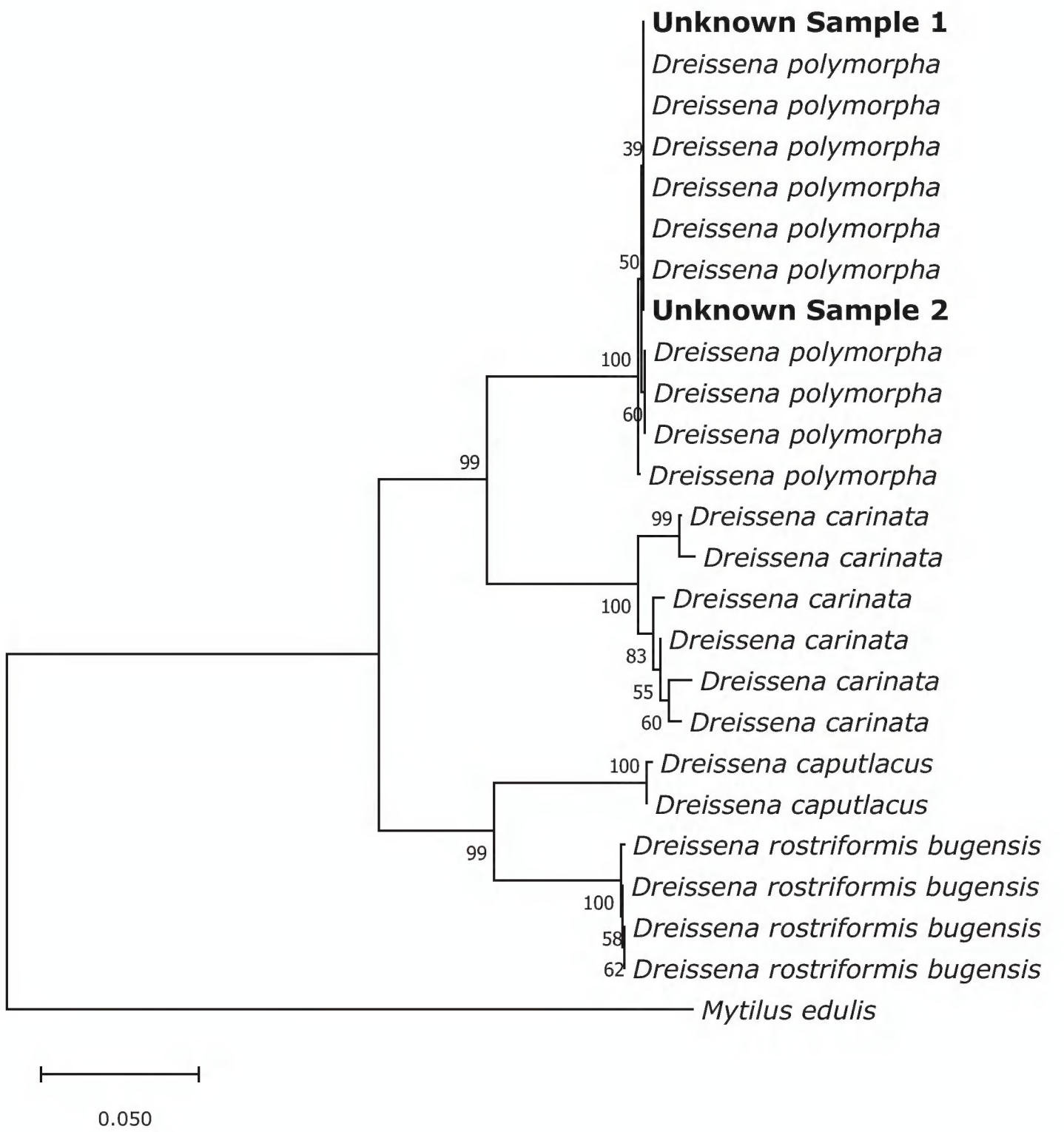

Figure 2. Neighbor joining tree from Mega. Bootstrap values are the percentage of trees supporting the shown topology with *Mytilus edulis* set as the root. The scale bar indicates the number of base differences per site. Unknown Sample 1 and 2 are the two mussels found attached to *Viviparus viviparus*. These results show that the unknown samples are *Dreissena polymorpha*.

PCR reactions were conducted in 10 μL volume reactions, containing 1 μL of forward and reverse primers (5mM concentration), template DNA, 10X PCR buffer (Invitrogen, USA) and dNTPs, 0.1 μL Taq DNA Polymerase (Invitrogen, USA) and 4.9 μL of nuclease-free water. Amplification was performed under the following conditions: 94 °C for 3 minutes; 35 cycles of 94 °C for 45 seconds, 48 °C for 45 seconds, and 72 °C for 60 seconds; 72 °C for 7 minutes. PCR products were sequenced on Sanger sequencing platform (Applied Biosystems, USA) at Eurofins Genomics (Kiel, Germany).

Sequencing results and analysis

Raw COI sequences were assembled and trimmed using CodonCode Aligner v 3.7.1 (Codon Code Corporation). Each sequence was blasted on NCBI (https://www.ncbi.nlm.nih.gov/) and BOLD (Ratnasingham and Hebert 2007). Sequences with ≥ 98% similarity were used as the preliminary identification results and implicated *D. polymorpha*. To verify the species identification, we constructed a phylogenetic tree by first downloading from BOLD ten sequences of *D. polymorpha*, two sequences of each additional *Dreissena* species found in BOLD (*D. carinata*, *D. rostriformis bugensis*, *D. caputlacus*), and one outgroup species (*Mytilus edulis*) (Suppl. material 1). All sequences were aligned using MUSCLE (Edgar 2004) in Unipro UGENE v37.0 (Okonechnikov et al. 2012). A phylogenetic tree was constructed using the neighbour-joining method and maximum likelihood in MEGA v10.1.8 (Kumar et al. 2018) with 10000 iterations, following default settings. The final analysis included 583 bases and 25 total sequences.

Alignments to databases of known samples (NCBI, BOLD) showed that the two mussels had high sequence similarity (> 98%) to *D. polymorpha*. Subsequent phylogenetic analysis further supported the assignment of these samples to *D. polymorpha*. Note that neighbour-joining and maximum likelihood (results not shown) revealed the same phylogenetic relationships. Therefore, we have high confidence that these samples are *D. polymorpha*.

Discussion

While concern surrounds the spread of commensal organisms, pathogens, and incidental organisms associated with non-native species in the pet trade (Patoka et al. 2020; Lozek et al. 2021; Stanicka et al. 2022), here we highlight the overlooked risk of native species in the trade facilitating the spread of non-native hitchhikers. *Viviparus viviparus* is a species distributed across Europe and advertised as being suitable for garden ponds. Escape from ponds is considered a major pathway for freshwater species introductions (Patoka et al. 2016), and accidental introductions of zebra mussels could lead to further dispersal through zoochorous means (Coughlan et al. 2017, 2019), or flooding events, as has been the case for aquaculture facilities in the past (Casimiro et al. 2018; McGlade et al. 2022). Indeed, questions surrounding the conditions under which *V. viviparus* were kept prior to shipping require answers, both to establish the biosecurity risk of that facility and to establish what other species in the pet and garden trade could be subject to similar hitchhiking. It may be that *V. viviparus* is unique as it is a European native capable of surviving the conditions under which it could come into contact with zebra mussels (i.e. it may have been held in outdoor ponds prior to collection and shipping), and in size, with a shell large enough for zebra mussels to attach to. However, zebra mussels have previously been found attached to the carapaces of crayfishes (Ďuriš et al. 2007), and the European native crayfish *Astacus astacus* is also sold for garden ponds in Germany and potentially held in outdoor stocking ponds

prior to shipment. With crayfish capable of overland dispersal and able to shed their mussel load upon moulting (Coughlan et al. 2017), the purchase of this species may pose an even greater risk of carrying zebra mussels and similar species (e.g. quagga mussels, *D. bugensis*).

Calls have been made for "white lists" of low-risk species that can be sold in the pet trade in place of risky species (Simberloff 2006; Patoka et al. 2018). However, we demonstrate that a native species (*V. viviparus*) can be a vector for ecologically detrimental invaders. Thus, even when a species itself is non-invasive and transported within its native range, and therefore immune to bans on trade stemming from legislation like the EU List of Union Concern, the potential for non-native hitchhikers can increase the ecological risk of nearly any traded organism. Indeed, Simberloff (2006) called for any white list species to be subject to "serious expert scrutiny" and we propose this should be the case for native species, with the ability to transport invasive species assessed within any potential risk assessments.

Greater biosecurity practices are also required, and need to be at the forefront of future policy revisions. The recommendations of Ložek et al. (2021), despite being focused on biosecurity measures surrounding the exportation of wild-caught individuals, could also prove effective in the case of species being held and/or bred in outdoor ponds. For example, the checking and disinfection of individuals collected from outdoor ponds, quarantining before transporting, and regular sanitation of outdoor stock ponds could help limit future incidental transport of invaders such as zebra mussels. Environmental DNA surveillance could be another solution for detecting invasive species at small abundances, as has been done effectively when assessing water samples from stores selling live bait for the DNA of invasive fish species (Nathan et al. 2015). Of course, species held in outdoor ponds could be host to other, unknown, less conspicuous hitchhikers, and the enforcement of intermittent stock health assessments could help limit the transportation of pathogens and parasites into novel ecosystems. Further, steps need to be taken to prevent the creation of more "dead letters", i.e. laws that exist but are not implemented (Patoka et al. 2018), and ensure enforcement. Beyond this, raising awareness amongst customers through a simple warning to check for unexpected organisms could provide an effective last line of defense.

Acknowledgements

JWED and EB conceived the study. JWED discovered the *D. polymorpha* and prepared the initial manuscript, with DNA sequencing performed by RSB and SSWC. All authors provided valuable input into the development of the final manuscript and have given approval for publication. JWED was supported by the Alexander von Humboldt Foundation. Thanks also to the editor Eric R. Larson, reviewer Ian Duggan and an anonymous reviewer for their constructive feedback and valuable recommendations for improving the manuscript.

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Supplementary material 1

List of species and accession numbers used for constructing phylogenetic trees

Authors: James W. E. Dickey, Reid S. Brennan, Sheena Suet-Wah Chung, Jonathan M. Jeschke, Gregor T. Steffen, Elizabeta Briski

Data type: table

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Link: https://doi.org/10.3897/neobiota.83.97647.suppl1

Supplementary material 2

FASTA file of all sequences used to generate the phylogenetic tree

Authors: James W. E. Dickey, Reid S. Brennan, Sheena Suet-Wah Chung, Jonathan M. Jeschke, Gregor T. Steffen, Elizabeta Briski

Data type: FASTA file

Explanation note: The two unknown mussel samples (here confirmed to be *D. polymorpha*) have been uploaded to NCBI GenBank under accession numbers OP714457 and OP714458.

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